In the Specification

Please enter the Abstract amended to read as follows:

Disclosed is a process for identifying clones having a specified activity of interest, which process comprises (i) generating one or more expression libraries derived from nucleic acid directly isolated from the environment; and (ii) screening said libraries utilizing a fluorescence activated cell sorter to identify said clones. More particularly, this is a process for identifying clones having a specified activity of interest by (i) generating one or more expression libraries derived from nucleic acid directly or indirectly isolated from the environment; (ii) exposing said libraries to a particular substrate or substrates of interest; and (iii) screening said exposed libraries utilizing a fluorescence activated cell sorter to identify clones which react with the substrate or substrates. Also provided is a process for identifying clones having a specified activity of interest by (i) generating one or more expression libraries derived from nucleic acid directly or indirectly isolated from the environment; and (ii) screening said exposed libraries utilizing an assay requiring co-encapsulation, a binding event or the covalent modification of a target, and a fluorescence activated cell sorter to identify positive clones.

Please amend Paragraph [0045] on page 16 as follows:

[0045] Figure 4 illustrates the protocol used in the cell sorting method of the invention to screen for recombinant enzymes, in this case using a [()]λ-library excised into *E. coli*. The expression clones of interest are isolated by sorting. The procedure is described in detail in Examples 1, 3 and 4.

Please amend paragraph [0082] on page 27 as follows:

[0082] Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, *e.g.*, the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), [()]α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium.

Please amend paragraph [0136] on page 38 as follows:

[0136] Lambda vectors can also accommodate relatively large DNA molecules, have high cloning and packaging efficiencies and are easy to handle and store compared to plasmid vectors.]([λ-ZAP vectors (Stratagene Cloning Systems, Inc.) have a convenient subcloning feature that allows clones in the vector to be excised with helper phage into the pBluescript phagemid, eliminating the time involved in subcloning. The cloning site in these vectors lies downstream of the *lac* promoter. This feature allows expression of genes whose endogenous promoter does not function in *E. coli*.

Please amend paragraph [0147] on page 41 as follows:

[0147] Several methods have been described for using reporter genes to measure gene expression. These reporter genes encode enzymes not ordinarily found in the type of cell being studied, and their unique activity is monitored to determine the degree of transcription. Nolan *et*

al., developed a technique to analyze β-galactosidase expression in mammalian cells employing fluorescein-di-[()β-D-galactopyranoside (FDG) as a substrate for β-galactosidase, which releases fluorescein, a product that can be detected by a fluorescence-activated cell sorter (FACS) upon hydrolysis (Nolan *et al.*, 1991). A problem with the use of FDG is that if the assay is performed at room temperature, the fluorescence leaks out of the positively stained cells. A similar problem was encountered in other studies of (galactosidase measurements in mammalian cells and yeast with FDG as well as other substrates (Nolan *et al*, 1988; Wittrup *et al.*, 1988). Performing the reaction at 0°C appreciably decreased the extent of this leakage of fluorescence (Nolan *et al.*, 1988). However this low temperature is not adaptable for screening for, for instance, high temperature β-galactosidases. Other fluorogenic substrates have been developed, such as 5-dodecanoylamino fluorescein di-[()β-D-galactopyranoside (C_{12}-FDG) (Molecular Probes) which differs from FDG in that it is a lipophilic fluorescein derivative that can easily cross most cell membranes under physiological culture conditions. The green fluorescent enzymatic hydrolysis product is retained for hours to days in the membrane of those cells that actively express the *lacZ* reporter gene. In animal cells C_{12}-FDG was a much better substrate, giving a signal which was 100 times higher than the one obtained with FDG (Plovins et al., 1994). However in Gram negative bacteria like *E. coli,* the outer membrane functions as a barrier for the lipophilic molecule C_{12}-FDG and it only passes through this barrier if the cells are dead or damaged (Plovins et al). The fact that C_{12} retains FDG substrate inside the cells indicates that the addition of unpolarized tails may be used for retaining substrate inside the cells with respect to other enzyme substrates.

Please amend paragraph [0149] on page 43 as follows:

[0149] The heat treatment of *E. coli* permeabilizes the cells to allow the substrate to pass through the membrane. Control strains containing plasmid DNA without insert and stained with the same procedure show no fluorescence. Phase contrast microscopy of heated cells reveals that cells maintain their structural integrity up to 2 hours if heated up to 70°C. The lipophilic tail of the modified fluorescein-di-[(] β-D-galactopyranoside prevents leakage of the molecule, even at elevated temperatures. The attachment of a lipophilic carbon chain changes the solubility of substrates tremendously. Thus, substrates containing lipophilic carbon chains can be generated and utilized as screening substrates in the present invention. For instance, the following activities may be detected utilized the indicated substrates. Different methods can be employed for loading substrate inside the cells. Additionally, DMSO can be used as solvent up to a concentration of 50% in water to dissolve and load substrates without significantly dropping the viability of *E. coli*. Enzyme activity and leakage can be monitored with fluorescence microscopy.

Please amend paragraph [0185] on page 54 as follows:

[0185] Probe nucleic acid sequences designed according to the method described above can also be utilized in the present invention to "enrich" a population for desirable clones. "Enrich", as utilized herein, means reducing the number and/or complexity of an original population of molecules. For example, probes are designed to identify specific polyketide sequences, and utilized to enrich for clones encoding polyketide pathways. [Figure X depicts in-situ hybridization of encapsulated fosmid clones with specific probes of interest, in this case] such as polyketide synthase gene probes. Fosmid libraries are generated in E.coli according to the methods described in the Example herein. Clones are encapsulated and grown to yield encapsulated clonal populations. Cells are lysed and neutralized, and exposed to the probe of

interest. Hybridization yields a positive fluorescent signal which can be sorted on a fluorescent cell sorter. Positives can be further screened via expression, or activity, screening. Thus, this aspect of the present invention facilitates the reduction of the complexity of the original population to enrich for desirable pathway clones. These clones can ~~the~~ then be utilized for further downstream screening. For example, these clones can be expressed to yield backbone structures (defined herein), which can [the] then be decorated in metabolically rich hosts, and finally screened for an activity of interest. Alternatively, clones can be expressed to yield small molecules directly, which can be screened for an activity of interest. Further more, multiple probes can be designed and utilized to allow "multiplex" screening and/or enrichment. "Multiplex" screening and/or enrichment as used herein means that one is screening and/or enriching for more than desirable outcome, simultaneously.

Please amend Paragraph [0189] on page 56 to read as follows:

[189] (Amended) *Streptomyces venezuelae*, unlike most other *Streptomyces* species, has been shown to sporulate in liquid [grown] growth culture. In some media, it also fragments into single cells when the cultures reach the end of vegetative growth. Because the production of most secondary metabolites, including bioactive small molecules, occurs at the end of log growth, it is possible to screen for *Streptomyces venezuelae* fragmented cells that are producing bioactivites by a fluorescence analyzer, such as a FACS machine, given the natural fluorescence of some small molecules.

Please amend Paragraph [0194] on page 57 to read as follows:

[194] (Amended) In the method of the present invention, the fluorescing properties of this and other similar compounds are utilized to screen for compounds of interest, as described

previously, or are utilized to enrich for the presence of compounds of interest. Host cells expressing recombinant clones potentially encoding gene pathways are screened for fluorescing properties. Thus, cells producing fluorescent proteins or metabolites can be identified. Pathway clones expressed in *E.coli* or other host cells, can yield bioactive compounds or "backbone structures" to bioactive compounds (which can subsequently be "decorated" in other host cells, for example, in metabolically rich organisms). The "backbone structure" is the fundamental structure that defines a particular class of small molecules. For example, a polyketide backbone will differ from that of a lactone, a glycoside or a peptide antibiotic. Within each class, variants are produced by the addition or subtraction of side groups or by rearrangement of ring structures ("decoration" or "decorated"). Ring structures present in aromatic bioactive compounds are known in some instance to yield a fluorescent signal, which can be utilized to distinguish these cells from the population. Certain of these structures can also provide absorbance characteristics which differ from the background absorbance of a non-recombinant host cell, and thus can allow one to distinguish these cells from the population, as well. Recombinant cells potentially producing bioactive compounds or "backbone" structures can be identified and separated from a population of cells, thus enriching the population for desirable cells. Thus, the method of the present invention also facilitates the discovery of novel aromatic compounds encoded by gene pathways, for example, encoded by polyketide genes, directly from environmental or other samples.

Please amend paragraph [0194] on page 57 as follows:

[0194] In the method of the present invention, the fluorescing properties of this and other similar compounds can [are] be utilized to screen for compounds of interest, as described previously, or are utilized to enrich for the presence of compounds of interest. Host cells

expressing recombinant clones potentially encoding gene pathways are screened for fluorescing properties. Thus, cells producing fluorescent proteins or metabolites can be identified. Pathway clones expressed in E.coli or other host cells, can yield bioactive compounds or "backbone structures" to bioactive compounds (which can subsequently be "decorated" in other host cells, for example, in metabolically rich organisms). The "backbone structure" is the fundamental structure that defines a particular class of small molecules. For example, a polyketide backbone will differ from that of a lactone, a glycoside or a peptide antibiotic. Within each class, variants are produced by the addition or subtraction of side groups or by rearrangement of ring structures ("decoration" or "decorated"). Ring structures present in aromatic bioactive compounds are known in some instance to yield a fluorescent signal, which can be utilized to distinguish these cells from the population. Certain of these structures can also provide absorbance characteristics which differ from the background absorbance of a non-recombinant host cell, and thus can allow one to distinguish these cells from the population, as well. Recombinant cells potentially producing bioactive compounds or "backbone" structures can be identified and separated from a population of cells, thus enriching the population for desirable cells. Thus, the method of the present invention also facilitates the discovery of novel aromatic compounds encoded by gene pathways, for example, encoded by polyketide genes, directly from environmental or other samples.

Please delete paragraph [0214] on page 64 and substitute the following paragraph:

[0214] Prepare the following ligation reactions (5 ml reactions) and incubate 4°C, overnight:

Sample	H_2O	10X Ligase Buffer	10mM rATP	Lambda arms (ZAP)	Insert DNA	T4 DNA Ligase (4 Wu/(l)
Fraction 1-4	0.5 ml	0.5 ml	0.5 ml	1.0 ml	2.0 ml	0.5 ml
Fraction 5-7	0.5 ml	0.5 ml	0.5 ml	1.0 ml	2.0 ml	0.5 ml

On pages 69 and 70, please delete the Cited Literature section and substitute the following:

--**[0234]** Alting-Mees, M.A., Short J.M., *Nucl. Acids. Res.* **1989,** 17, 9494.

[0235] Hay, B. and Short, J. *Strategies,* **1992**, 5, 16.

[0236] Enzyme Systems Products, Dublin CA 94568; Molecular Probes, Eugene, OR 97402, Peninsula Laboratories, Belmont, CA 94002.

[0237] Adams, M.W.W., Kelly, R.M., *Chemical and Engineering News*, **1995**, Dec. 18.

[0238] Amann, R., Ludwig, W., and Schleifer, K.-H. *Microbiological Reviews*, **1995**, 59, 143.

[0239] Barnes, S.M., Fundyga, R.E., Jeffries, M.W. and Pace, N.R. *Proc.Nat. Acad. Sci. USA* , **1994**, 91, 1609.

[0240] Bateson M. M., Wiegel, J., Ward, D. M., *System. Appl. Microbiol.* **1989**, 12, 1-7

[0241] Betz, J. W., Aretz, W., Hartel, W., *Cytometry*, **1984**, 5, 145-150

[0242] Davey, H. M., Kell, D. B., *Microbiological Reviews*, **1996**, 60, 4, 641-696

[0243] Diaper, J. P., Edwards, C., *J. Appl. Bacteriol.*, **1994**, 77, 221-228

[0244] Enzyme Nomenclature, *Academic Press*: NY, **1992**.

[0245] Faber, Biotransformation in organic chemistry 2nd edition, *Springer Verlag*, **1995**.

[0246] Faber, U.S. Tonkovich and Gerber, Dept. of Energy Study, **1995**.

[0247] Fiering, S. N., Roeder, M., Nolan, G. P., Micklem, D. R., Parcks, D. R., Herzenberg, L. A. *Cytometry*, **1991**, 12, 291-301.

[0248] Giovannoni, S. J., Britschgi, T. B., Mover, C. L., Field, K. G., *Nature*, **1990** 345, 60-63.

[0249] Murray, M. G., and Thompson, W. F., *Nucl. Acids Res.*, **1980**, 8, 4321-4325.

[0250] Nolan, G. P., Fiering, S., Nicolas, J., F., Herzenberg, L. A., *Proc. Natl. Acad. Sci. USA*, **1988**, 85, 2603-2607.

[0251] Plovins A., Alvarez A. M., Ibanez M., Molina M., Nombela C., Appl. Environ. Microbiol., **1994**, 60, 4638-4641.

[0252] Short, J.M., Fernandez, J.F. Sorge, J.A., and Huse, W. *Nucleic Acids Res.*, **1988**, 16, 7583-7600.

[0253] Short, J.M., and Sorge, J.A. *Methods in Enzymology*, **1992**, 216, 495-508.

[0254] Tonkovich, A., L., Gerber, M. A., US Department of Energy, Office of Industrial Technology, Biological and Chemical Technologies Research Program under contract DE-AC06-76RLO 1830. --

[0255] Torvsik, V. Goksoyr, J. Daae, F. L., *Appl. And Environm. Microbiol.* **1990**, 56, 782-787.

[0256] Wittrup, K. D., Bailey, J. E., Cytometry, **1988**, 9, 394-404.

[0257] Wrotnowski, *Genetic Engeneering News*, **1997**, Feb. 1.

Please substitute the following new title for the invention on page 1 of the Specification:

--A METHOD FOR HIGH THROUGHPUT SCREENING OF AN ENVIRONMENTAL LIBRARY--